METHODOLOGY

Study type: observational study

Study design: Cross sectional study

Study duration: Aug 2007- March 2011

Source of Data collection: KLE City Polyclinic and Diagnostic center, Samadevi Galli, Belgaum, Karnataka State, India.

Sample size: Total 400 (200 diabetic patients and 200 non diabetic individuals)

Calculation of sample size:

Sample size for screening test was assessed, based on the formula

$$\mathbf{n= \frac{4\ Z_{\alpha}^{2}\ pq}{d^{2}}}$$

P : sensitivity of screening test (p value assumed to be 80%)

q: 100- p% q=10%

d: Error (10% of relative error)

$\mathbf{Z_{\alpha}=1.}$96 for 95% confidence.

$$\mathbf{N= \frac{4 \times 2^{2} \times 80 \times 20}{8^{2}} =400.}$$

400 = 200 individuals with disease (diabetic),

200 individuals without disease (non diabetic).

Sampling design: Non probability sampling

Sampling method: Consecutive sampling (those met the inclusion criteria).

Study participants: Subjects who reported to the laboratory for blood glucose analysis, during study period as per inclusion and exclusion criteria.

Instruments: Micropipettes, Semi automated machine, Erba CHEM – 5 Plus V2), reagent Erba Glucose Kit, Trinders method, end point.

Materials used: Structured proforma, consent form, Glucose reagent kit (Liquixx company)

Inclusion criteria for diabetic patients: Patients with confirmed diabetes.

Exclusion criteria: Presence of any obvious oral lesions.

Patients treated for any salivary gland disorders.

Patients on medication for any other local or systemic disease other than diabetes mellitus and hypertension.

Procedure:

After obtaining Institutional ethical clearance the study was carried out prospectively between 2007 August -2011 Feb in K.L.E'S Polyclinic & Diagnostic Centre, Samadevi Galli, Belgaum. A total of 200 type 2 diabetic patients and 200 healthy subjects were included in the study. All subjects were from same geographic area. A written informed consent was obtained from all the subjects.

Estimation of fasting and post prandial serum glucose levels and salivary glucose levels were carried out for these subjects.

Case history and consent form: A detailed case history was recorded as per the proforma attached. Patient's history regarding duration of the disease, type of glycemic control, family history and personal history were recorded. Patients were briefed regarding the study and their enrolment, for which a written consent was obtained. Patients blood & saliva were collected in fasting and postprandial state.

For fasting sample: 10-12 hours following fast.

For post prandial sample : 2 hours after meal.

Collection of blood: Under aseptic conditions using a sterile disposable 25 gauge needle, intravenous blood was collected from the median vein. The blood was allowed to clot in the test tube. Centrifuged at 3000 rpm for 10 minutes and then serum was separated.

Collection of Saliva: The patients were asked to rinse their mouth thoroughly with water. Unstimulated whole saliva was collected in a sterile container by asking the patient to expectorate into it gradually over period of 5-10 minutes till approximately 1 ml of saliva was collected. Sample was transferred to a disposable test tube and centrifuged at 2000 rpm for 2-3 minutes and supernatant was separated.

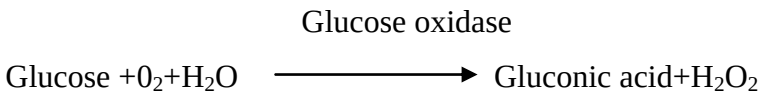
Assay Procedure(end point):

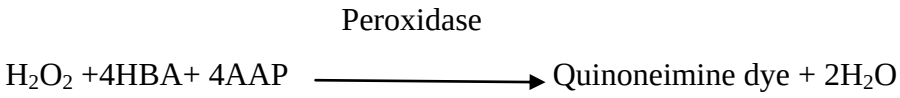
Pipette into test tube labelled as	**Blank**	**Standard**	**Test**
Sample	--	--	10µl
Standard	--	10µl	--
Enzyme reagent	1.0ml	1.0ml	1.0ml

After every addition, the sample was mixed well and incubated at 37^0c for 5 minutes. The absorbance of standard and test against blank was noted at 505-670 nm on semi automated machine.

Assay principle:

Glucose Oxidase oxidize glucose to gluconic acid and hydrogen peroxide.In presence of enzyme peroxidase, released hydrogen peroxide is coupled with Phenol and 4-Aminoantipyrine (4-AAP) to form coloured Quinoneimine dye. Absorbance of coloured dye is measured at 505 nm and is directly proportional to glucose concentration in the sample.





4AAP : 4 - amino antipyrine

4HBA : 4 – Hydroxy benzoic acid

The obtained results were tabulated separately for diabetic and non diabetic group.

Statistical Analysis:

The data was entered separately for diabetic and non diabetic individuals on excel spread sheet, tabulated and subjected to statistical analysis by using SPSS software. The mean, Standard deviation, degree of freedom (DF) and P value were assessed for Age, Blood glucose levels in fasting and post prandial state, salivary glucose levels in fasting and post prandial state.

The sensitivity, specificity, negative predictive value and positive predictive value were analyzed at different salivary glucose levels. This was done in order to determine the use of salivary glucose levels as screening mode in post prandial state and in fasting state.